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PATHOLOGIC CHANGES INDUCED BY COAL-FIRED FLY ASH IN HAMSTER TRACHEAL GRAFTS*

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SUMMARY

The toxicity of fly ash from a coal-fired power plant for respiratory tract epithelium was studied in heterotropic tracheal grafts. Hamster tracheal grafts were continuously exposed to beeswax-cholesterol pellets containing 100, 1000 and 5000 μg fly ash and evaluated at 1, 2, 4 and 14—15 months of exposure. Histopathologic effects and the autoradiographic pattern of [^{3}H]thymidine incorporation were determined. In all concentrations of fly ash, an early mild submucosal inflammatory response was seen. Morphologic response of the tracheal epithelium was characterized by hyperplasia followed by squamous metaplasia and atrophic lesions. Although a rare papillomatous structure with cellular atypia was seen in grafts receiving 1000 μg fly ash, no carcinomas appeared during the 15-month observation period. Varying degrees of submucosal toxicity were also observed during this time period. Autoradiographic studies showed a significant increase in [^{3}H]thymidine incorporation in grafts receiving fly ash treatment.

Key words: Hamster tracheal grafts; Fly ash; Respiratory tract mucosa; Chronic toxicity

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INTRODUCTION

Fly ash is a complex particle showing a high degree of chemical and physical heterogenity [1,2]. These properties make it difficult to predict the biological effects of fly ash on conducting airways. In a previous paper [3], we described the effects of fly ash from a coal-fired power plant on differentiated tracheal epithelium. Our results showed a correlation between fly ash-induced changes in tracheobronchial epithelium of hamsters exposed in vivo and effects observed in hamster tracheal epithelium exposed in organ culture. Tracheal organ cultures, however, do not permit us to examine chronic effects of fly ash on the mucosa of the conducting airways. Conventional in vivo approaches [4,5] to the study of respiratory tract toxicity have major drawbacks because the dose can not be controlled nor can the target site be defined. We, therefore, chose the tracheal transplant model of Griesemer et al [6]. With this system, the tracheal mucosa can be continuously exposed to fly ash particles with slow release of the material from the vehicle.

In the present study, we report the biological effects of coal-fired fly ash in heterotopic hamster tracheal transplants. Mucosal and submucosal changes resulting from continuous exposure to fly ash was monitored at various times over an exposure period of 15 months. Autoradiographic investigations were also carried out to determine changes in epithelial proliferation.

MATERIALS AND METHODS

Tracheal transplants

Male carcinogen-susceptible F_1D hybrid, B10 F_1D Alexander hamsters (Bio-Research Consultants, Inc., Cambridge, MA) weighing 75–125 g were used as trachea donors and recipients for this study. A total of 144 hamsters were used as trachea donors. Tracheas were aseptically removed, washed in L-15 medium and tied to 3-cm long sterile polyethylene tubing. Two tracheas were transplanted subcutaneously into the retroscapular region of each 2-month-old isogenic recipient hamster [6].

Preparation of fly ash pellets

Fly ash and control pellets without fly ash were prepared in a similar manner as described by Pal et al. [7]. Fly ash was obtained from an electrostatic precipitator (ESP) hopper collected from a coal-fueled power plant using eastern Kentucky coal [8]. The ESP hopper ash was a 0–3.3 μm aerodynamic diameter fraction, prepared from ash less than 150 μm (geometric). The particles were fractionated with a Bahco microparticle classifier (Harry Dietert Co., Detroit, MI) in an atmosphere of low relative humidity (<30%) [8]. Fly ash was incorporated into beeswax: cholesterol (1:9) mixture by heating to 120°C, mixing while hot on a vortex mixer and the mixture formed into cylindrical pellets in a preheated pellet mold designed after Griesemer et al. [6].

Morphological and autoradiographical assessment of the effects of fly ash

Two weeks after transplantation, when tracheas showed early vascularization, tracheas were implanted with beeswax/cholesterol pellets containing 100, 1000 or 5000 μg of fly ash. For each concentration, 32 transplanted tracheas were used. After 1, 2, 4 and 14—15 months of exposure, 8 tracheal transplants from each fly ash concentration were sampled for histological evaluation and epithelial proliferation activity. Six tracheal transplants exposed to beeswax/cholesterol pellets without fly ash and untreated transplants were taken at the same intervals. Four hours before killing, recipient hamsters received an intraperitoneal injection of [*methyl*-^{3}H]thymidine (spec. act., 20 Ci/mmol, Research Products International Corp., Elk Grove Village, IL) in a dosage of 0.5 μCi/g body weight.

Tissues were fixed in 10% neutral buffered formalin. Using a sharp razor blade, each trachea was divided into 2 equal portions. The 2 portions of trachea were embedded close together making sure they were level and standing so that transverse sections could be microtomed. Five step-sections, 100 μm apart, were made. A total of 10 paraffin cross-sections, 5-μm each, from the middle of each trachea were made and stained with hematoxylin and eosin (H & E). Sections were analyzed according to histological changes in the epithelium and submucosa. All sections were examined microscopically in a blind-random fashion. For autoradiography, 5-μm sections were deparaffinized in xylene and dipped in Kodak NTB2 nuclear tract emulsion. After drying, slides were placed in a light-tight box and exposed at 4°C for 1 week. The autoradiographs were developed in Kodak D-19 developer and stained with H & E. The number of epithelial cells with 5 grains or more over their nuclei were counted from each trachea. The labeling index was determined by calculating the ratio of labeled cells in a population per total cells in that population.

RESULTS

Histopathologic effects

Morphological changes in tracheal transplants receiving various concentrations of fly ash or control pellets are summarized in Table I. All 3 concentrations of fly ash caused hyperplasia of the epithelial lining at the end of 1 month exposure (covering 25—75% of the total epithelium). By 2 months, the hyperplastic reaction had lessened (10–25% of the epithelium). Foci of squamous metaplasia was observed in some tracheas at each of the 3 dose levels of fly ash-treated transplants (5—20% of the surface area) but was more extensive in higher dose levels. Atrophic-pleomorphic lesions were frequently observed (25—50% of the total epithelium) at the 2-, 4-, and 14–15-month exposure periods. At 14—15 months a considerable portion of these atrophic-pleomorphic lesions showed cellular atypia (approx. 75%). Fly ash particles could not be seen in the lumen of these grafts.

Tracheal grafts exposed to beeswax pellets without fly ash (vehicle control) showed a moderate columnar cell hyperplasia at the 1-, 2- and

TABLE I

HISTOPATHOLOGICAL CHANGES IN TRACHEAL GRAFTS EXPOSED UP TO 15 MONTHS TO COAL-FIRED FLY ASH

Treatment	No. tracheas showing histopathological changes at months after initiation of fly ash															
	Hyperplasia				Squamous metaplasia				Atrophic-pleomorphic lesions[a]				Submucosal lesisons[b]			
	1	2	4	14—15	1	2	4	14—15	1	2	4	14—15	1	2	4	14—15
Fly ash pellets																
100 μg	4/8	3/8	3/8	2/8	0/8	3/8	2/8	2/8	0/8	2/8	2/8	4/8	0/8	2/8	2/8	3/8
1000 μg	7/8	3/8	3/8	3/8	2/8	4/8	3/8	2/8	0/8	5/8	4/8	5/8	4/8	3/8	3/8	4/8
5000 μg	8/8	5/8	4/8	3/8	3/8	4/8	3/8	3/8	0/8	8/8	7/8	7/8	3/8	3/8	4/8	4/8
Pellets without fly ash																
(Vehicle Control)	3/6	2/6	2/6	1/6	0/6	1/6	1/6	0/6	0/6	0/6	1/6	1/6	0/6	1/6	0/6	1/6
Untreated Transplant	0/6	1/6	0/6	0/6	1/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6

[a] Atrophic-pleomorphic lesions defined as flattening and attenuation of the epithelial lining with loss of differentiative features and varying degrees of cellular atypia [9].

[b] Submucosal lesions consisted of granulation and resorption of foreign bodies, proliferation of fibroblasts, fibrosis and presarcomatous lesions.

4-month sampling periods. Tracheal transplants without pellets (untreated control) showed no toxic response. From the second month until the end of the experiment, the epithelium consisted of a low cuboidal mucocillary epithelium.

One out of 8 transplants receiving 1000 μg/pellet developed papillomatous structures with cellular atypia. No carcinomas occurred in any of the fly ash-treated groups or control groups. A sarcoma did arise in the granulation tissue surrounding the polyethylene tubing in a pellet control trachea.

Tritiated thymidine incorporation

The labeling index of tracheal epithelial cells after various periods following initiation of treatment with fly ash are given in Table II. After 1 month, the labeling index of the untreated control transplants was approximately 2%. This represents a substantial increase compared to a labeling index of 0.1% for the mucosa of normal recipient hamster trachea. The number of [^{3}H]thymidine incorporating cells in untreated controls remained at 1% through 15 months. Tracheal grafts exposed to beeswax pellets without fly ash showed only a slight increase in [^{3}H]thymidine incorporation. Fly ash-treated tracheal grafts removed at 1 month showed a marked increase in cells in DNA synthesis, as the concentration of fly ash increased. After 2, 4 and 14–15 months, [^{3}H]thymidine incorporation in grafts receiving fly ash treatment remained higher than controls.

DISCUSSION

In a previous paper, we have shown a correlation between coal-fired fly ash-induced changes in tracheal epithelium of hamsters exposed in organ

TABLE II

LABELING INDEX OF EPITHELIAL CELLS FROM TRACHEAL GRAFTS CONTINUOUSLY EXPOSED TO VARIOUS CONCENTRATIONS OF FLY ASH[a]

Time after initiation of exposure (months)	Treatment				
	Untreated transplants	Pellets without fly ash	Fly ash pellets		
			100 μg	1000 μg	5000 μg
1	2.3 ± 0.4[b]	2.7 ± 0.6	3.5 ± 1.1	6.6 ± 1.4[c]	7.8 ± 1.9[c]
2	0.9 ± 0.1	1.3 ± 0.3	3.2 ± 0.7[c]	4.1 ± 1.0[c]	6.3 ± 1.5[c]
4	1.0 ± 0.3	1.4 ± 0.4	2.9 ± 0.6[c]	3.8 ± 0.7[c]	5.8 ± 0.9[c]
14–15	0.6 ± 0.2	1.2 ± 0.5	2.6 ± 0.7	4.1 ± 0.9[c]	4.4 ± 1.1[c]

[a] Number of labeled cells/100 cells were counted on 400–800 epithelial cells from the mucosa of each tracheal graft.

[b] Mean value from 8 tracheal grafts ± S.E.

[c] For a given time after exposure, this labeling index in tracheal epithelial cells from grafts containing fly ash pellets is different from grafts containing pellets without fly ash (<0.05). Individual t-tests with Bonferroni correction were used [13].

culture and tracheobronchial epithelium of hamsters exposed in vivo [3]. Epithelial changes were characterized by basal cell hyperplasia and stratification, progressing to cornified epidermoid metaplasia. Since the time in which tracheal mucosa can be maintained in organ culture is limited, we have chosen the tracheal graft system [6] for investigating the chronic effects of fly ash on respiratory tract mucosa.

All 3 concentrations (100, 1000 and 5000 μg/pellet) of fly ash produced toxic changes in the tracheal mucosal epithelium and submucosa, although to varying degrees. After early hyperplasia, squamous metaplasia appeared, followed by atrophic-pleomorphic lesions. Epithelial cell proliferation was observed throughout the 14–15-month test period, indicating that the replicative capacity of the epithelium was maintained.

Fly ash particles represent a complex mixture of organic and inorganic compounds [8,10]. Thus studies of this complex mixture is more complicated than studies with single compounds. The amount of a single agent, such as benzo[*a*]pyrene (BaP) can be measured either by analysis of the material remaining in the pellet [9,11] or analysis of the target tissue for BaP and its metabolites [12]. Thus the biological effect can be related to the concentration of BaP. However, with a complex mixture such as fly ash the biological response of respiratory mucosa could be due to the interaction with whole fly ash particles or selective release of individual chemical components. Although it is difficult to define the constituents responsible for the toxic alterations in the respiratory mucosa, tracheal grafts have provided a suitable model for chronic studies of fly ash effects.

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